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The role of HIV-associated vasculopathy in the etiology of ischemic stroke

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Abstract:	<p>Background: HIV infection is a recognized risk factor for young stroke but the exact mechanisms are poorly understood. We studied the clinical, radiologic and histologic features of HIV-related ischemic stroke to gain insight into the disease mechanisms.</p> <p>Methods: We conducted a prospective, in-depth analysis of adult ischemic stroke patients presenting to Queen Elizabeth Central Hospital, Blantyre, Malawi, in 2011.</p>

	<p>Results: We recruited 64 HIV positive and 107 HIV negative patients. Those with HIV were significantly younger ($p < 0.001$) and less likely to have established vascular risk factors. Patients with HIV were more likely to have large artery disease (21% versus 10%; $p < 0.001$). The commonest etiology was HIV-associated vasculopathy (24 [38%]), followed by opportunistic infections (16[25%]). Sixteen out of 64 (25%) had a stroke soon after starting antiretroviral therapy (ART), suggesting an immune reconstitution-like syndrome. In this group CD4+ T-lymphocyte count was low, despite a significantly lower HIV viral load in those recently started on treatment ($p < 0.001$).</p> <p>Conclusions: HIV-associated vasculopathy and opportunistic infections are common causes of HIV-related ischemic stroke. Furthermore, subtypes of HIV-associated vasculopathy may manifest as a result of an immune reconstitution-like syndrome after starting ART. A better understanding of this mechanism may point towards new treatments.</p>
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Title:

The role of HIV-associated vasculopathy in the etiology of ischemic stroke

Running Title:

Etiology of HIV ischemic stroke

40-word summary:

The etiologic spectrum of stroke is different in those with HIV infection compared to those without. HIV-associated vasculopathy was the commonest mechanism found. We describe an immune reconstitution syndrome-like vasculopathy among those starting ART.

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Abstract

Background: HIV infection is a recognized risk factor for young stroke but the exact mechanisms are poorly understood. We studied the clinical, radiologic and histologic features of HIV-related ischemic stroke to gain insight into the disease mechanisms.

Methods: We conducted a prospective, in-depth analysis of adult ischemic stroke patients presenting to Queen Elizabeth Central Hospital, Blantyre, Malawi, in 2011.

Results: We recruited 64 HIV positive and 107 HIV negative patients. Those with HIV were significantly younger ($p < 0.001$) and less likely to have established vascular risk factors. Patients with HIV were more likely to have large artery disease (21% versus 10%; $p < 0.001$). The commonest etiology was HIV-associated vasculopathy (24 [38%]), followed by opportunistic infections (16[25%]). Sixteen out of 64 (25%) had a stroke soon after starting antiretroviral therapy (ART), suggesting an immune reconstitution-like syndrome. In this group CD4+ T-lymphocyte count was low, despite a significantly lower HIV viral load in those recently started on treatment ($p < 0.001$).

Conclusions: HIV-associated vasculopathy and opportunistic infections are common causes of HIV-related ischemic stroke. Furthermore, subtypes of HIV-associated vasculopathy may manifest as a result of an immune reconstitution-like syndrome after starting ART. A better understanding of this mechanism may point towards new treatments.

Key words: Stroke, Vasculopathy, HIV, Africa, Immune reconstitution syndrome

Introduction

Stroke incidence in low-to-middle income countries is increasing, especially in young populations.[1] In many of these regions, HIV is prevalent, and younger populations are more likely to have infectious causes of stroke.[2]

We recently showed that HIV infection makes a major contribution to the overall stroke burden (Population Attributable Fraction 15%) in Malawi.[2] It was the second leading risk factor overall (behind hypertension), and the most important among young stroke patients (Population Attributable Fraction 42%). Starting antiretroviral (ART) treatment appeared to contribute to stroke risk in the very immunosuppressed, but the mechanism of this is unknown.[2] Previous reports have shown that opportunistic infections, coagulopathy and cardio-thromboembolism are important etiologies to consider.[3] In addition, HIV infection may directly lead to HIV-associated vasculopathy via inflammatory intermediaries.[4] The term vasculopathy is defined as intimal hyperplasia more than expected for age, and thus encompasses several pathologic phenotypes of stroke found in HIV infection including, 1) HIV associated accelerated atherosclerosis, 2) non-atherosclerotic vasculopathy (patients have non-vasculitic abnormalities, with intimal hyperplasia that can progress to stenosis or aneurysmal dilatation) 3) HIV-associated vasculitis, and 4) small vessel disease.[5] Our understanding of the pathologic mechanisms of these phenotypes is incomplete. We have previously described more detailed clinico-pathologic classification of HIV-associated vasculopathy.[5]

Here we report the clinical, laboratory, radiologic and autopsy features of HIV ischemic stroke patients, explore how they differ from the non-HIV ischemic stroke population, and consider the mechanisms of stroke among those starting antiretroviral therapy (ART).

Methods

169 Participants

170 The study was conducted at the Queen Elizabeth Central Hospital, Blantyre Malawi; a large
 171 government hospital for much of Southern Malawi. The national prevalence of HIV in adults is 10.6%
 172 but higher (20%) in Blantyre.[6] Adults (age ≥ 18 years) who presented to the hospital within seven
 173 days of symptom onset, and met the WHO case definition of stroke 'a clinical syndrome consisting of
 174 rapidly developing clinical signs of focal (or global in case of coma) disturbance of cerebral function
 175 lasting more than 24 hours or leading to death with no apparent cause other than a vascular
 176 origin.',[7] were recruited to the study between February 2011 and April 2012 (Figure 1).

177

178 Procedure and etiological definitions

179 Clinical features, and potential vascular risk factors (i.e. age, gender, family history, ART use,
 180 hypertension, diabetes, hypercholesterolemia, acute infection, abdominal obesity, alcohol, smoking,
 181 substance use, and previous stroke/transient ischemic attack) were recorded. Stroke severity at
 182 baseline was assessed with the National Institutes of Health Stroke Scale (NIH stroke scale), and
 183 performed within 7 days of symptom onset.[8, 9] Evidence of peripheral vascular disease was
 184 determined by measuring ankle brachial index using a handheld vascular Doppler (HI Dop; Ana Wiz
 185 Ltd UK).[10] Magnetic Resonance Imaging (MRI) of the brain was performed within 7-days of
 186 admission. The definitions for risk factors, severity of stroke and imaging protocol were previously
 187 reported.[2]

188

189 Investigations included full blood count, total cholesterol, random glucose, HIV-1 serology and viral
 190 load, CD4+ T-lymphocyte count (CD4+ count) using previously described methods.[2] HIV-1 RNA load
 191 was measured using the Hologic Aptima HIV-1 Quant Dx assay (Hologic Inc, Manchester, UK). This
 192 sensitive assay has a lower limit of quantitation (LLOQ) of 30 copies/ml and a limit of detection of 13

copies/ml. .. Antiphospholipid syndrome diagnostics (anti-cardiolipin antibody, lupus anti-coagulant, anti- β_2 -glycoprotein 1; Cambridge Life Sciences Ltd, Cambridgeshire, UK), and specific infection screening (1. serum syphilis treponemal immunoassay + agglutination test and non-treponemal tests, and if positive cerebrospinal fluid [CSF] venereal disease research laboratory test, and 2. monoclonal intrathecal varicella zoster[VZV]-antibody determination)[11] were not done locally and thus, performed using standard protocols, at the haematology department, Royal Liverpool Hospital UK, and Public Health England respectively. All blood cultures and CSF diagnostics [i.e. microscopy, biochemistry, India ink and acid fast bacilli stains, cryptococcal antigen, standard bacterial culture, Mycobacterium tuberculosis (TB) culture] were performed locally at the Malawi-Liverpool-Wellcome Trust (MLW) laboratory. MLW laboratory participates in internationally recognised quality control programmes including UK National External Quality Assessment Service and the South African National Health Laboratory Service scheme. Chest X-ray, electrocardiogram, carotid/vertebral duplex ultrasound and echocardiography were also performed. When possible, a brain-only autopsy was performed in deceased HIV positive patients. Brain tissue was stored in 10% formalin and processed at the University of Edinburgh, UK. The tissue sections were stained with hematoxylin and eosin and Ziehl-Neelsen stain. Additional staining included p24 antigen (for HIV) and CD8, CD68 and CD3 antibodies (for inflammation). The results were interpreted by a neuropathologist and general pathologist with expertise in HIV infection. Although the pathologists were not blinded to the HIV status, a consensus had to be reached among these senior pathologists, with the third (HIV pathologist) arbitrating when needed.

The etiology was determined using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification (Figure 1). To expand the other determined and undetermined category and handle multiple etiologies in the HIV cohort, we also used the HIV Etiology for ischemic Stroke classification, as described previously (Figure 1). The physician that determined the final diagnosis was blinded to ART status, degree of immunosuppression and HIV viral burden.

218

219 Ethical consideration

220 The study was approved by the Liverpool School of Tropical Medicine, UK and the College of
221 Medicine Research Ethics Committee, University of Malawi. All participants or guardians gave
222 written informed consent.

223

224 Statistical analysis

225 Discrete variables were reported as absolute counts and percentages. Continuous variables as the
226 median with inter-quartile ranges. HIV viral load below the lower limit of quantitation were coded as
227 30 copies/ml (the assays quantification limit). This was then log transformed to compare the median
228 HIV viral load across specified groups. Contingency tables comparing 1) HIV positive/negative, and 2)
229 HIV etiology for stroke classification, were analyzed with Fisher's two-sided exact test. Kruskal-Wallis
230 non-parametric ANOVA was used to compare continuous variables.

231 Statistical analyses were done with STATA 11.2. and GraphPad Prism version 6, GraphPad Software
232 Inc., California, USA. A significance level of <0.05 was used throughout.

233

234

235 Results

236 Three hundred patients with suspected stroke were screened during the study period, of whom 171
 237 (64 HIV positive and 107 HIV negative) had ischemic strokes and met the entry criteria (Figure 1).
 238 Table 1 describes the demographic and clinical characteristics of the HIV positive and negative
 239 individuals. The 64 HIV-infected ischemic stroke cases form the basis of this study. Of these, 26
 240 (40%) were on ART and four had brain autopsy.

241

242 Comparison between HIV positive and negative patients

243 Compared with the HIV-negative patients, the HIV positive patients were significantly younger
 244 (median 40-years versus 66-years; $p < 0.001$), and less likely to be hypertensive (42% versus 89%;
 245 $p < 0.001$; Table 1). Other vascular risk factors including diabetes, hypercholesterolemia, and being a
 246 smoker, was more common in patients who were HIV negative. Imaging analysis showed that basal
 247 ganglia ischemia occurred more often with HIV ischemic stroke (68% versus 42%; $p < 0.001$). Using the
 248 TOAST classification, the other determined and undetermined etiologic category were common in
 249 both groups. However, for the better characterised categories, large artery disease had a different
 250 distribution and occurred more frequently in the HIV positive group (21% versus 10%; Table 1).

251

252 Etiology of HIV-related ischemic stroke

253 HIV-associated vasculopathy (i.e. accelerated atherosclerosis, non-atherosclerotic vasculopathy, HIV-
 254 associated vasculitis and small vessel disease) was the commonest etiology (38%), followed by
 255 opportunistic infections (25%); the latter included VZV, TB, and syphilis (Table 2). Although VZV was
 256 the most frequent infection, it was often not clinically obvious with only 3/9 (30%) having had a
 257 vesicular rash in the corresponding cranial distribution within 6 months of their stroke. No patient

had evidence of occult cryptococcal disease. Despite a comprehensive work-up, a specific cause was not determined in the 17% with cryptogenic stroke.

Age ($p<0.001$), CD4+ count ($p=0.031$), and ART status ($p=0.048$) differed significantly for the different etiologic groups. For example, non-atherosclerotic vasculopathy, HIV-associated vasculitis, opportunistic infection and cryptogenic stroke had a median age ≤ 45 yrs (i.e. young stroke) whereas those with atherosclerotic vasculopathy and cardio-thromboembolism were older (Table 3). In the most immunosuppressed patients whose median CD4+ count was <200 cell/mm³, HIV-associated vasculitis, opportunistic infections and antiphospholipid syndrome were the most frequently found etiologies. The ART status differed significantly for these patients: 67% of those with HIV-associated vasculitis had started ART in the 6 months prior to their stroke; in contrast, only 33% of those with antiphospholipid syndrome, and 19% of those with opportunistic infections had recently started ART (Table 3). Furthermore, blood HIV viral load differed across these groups, being high (median 3.5 and 4.7 copies/ml respectively) in patients with opportunistic infections and antiphospholipid syndrome, in contrast to being below the lower limit of quantitation, in patients with HIV-associated vasculitis. There were no significant differences in measured CSF cell count and biochemistry across the groups.

HIV-associated vasculopathy

The HIV-associated vasculopathy subtypes included accelerated atherosclerosis ($n=7$), non-atherosclerosis ($n=7$) and HIV-associated vasculitis ($n=9$); the median age for these patients was 60-years, 33-years and 35-years respectively (Table 3). Because there was only one case of small vessel disease, this was not included in the detailed analysis. The various types of HIV-associated vasculopathy differed by ART status and CD4+ count (Table 3); for example, no patient with

atherosclerotic vasculopathy had started ART in the last six months, compared with 43% of the non-atherosclerotic and 67% of the HIV-associated vasculitis subtypes.

At autopsy (Supplement Figure 1), two patients showed extensive atherosclerosis in all sized vessels (the images were consistent), one was on ART and young (50-years), with no established vascular risk factors. Although the other patient was older (74-years), not on ART, with a new diagnosis of hypertension; the degree of atherosclerosis was marked. All patients with HIV-associated vasculitis had a median CD4+ count of $<200\text{cells/mm}^3$, and HIV-1 RNA were below the lower limit of quantitation ; this differed from those with non-atherosclerotic and atherosclerotic vasculopathy (Table 3).

Initiating ART

Sixteen out of 64 (25%) patients had an ischemic stroke within 6 months of starting ART. Ten (63%) of these recent ART-initiators had a stroke within 1 month of starting ART. The median age, CD4+ count and blood HIV viral load were 37-years (IQR:31,47), 122 cells/mm^3 (IQR:73,237) and $1.5\log_{10}$ copies/ml (IQR:0.7,2.1) respectively. We explored established risk factors for immune reconstitution inflammatory syndrome (IRIS), such as anemia, low CD4+ count, and a drop in HIV viral load. Patients recently started on ART had the lowest median CD4+ count (122 [IQR: 73,236] compared to 159 [IQR: 65,279] in patients never started on ART, and 295 [IQR: 192,455] in patients on ART for ≥ 6 months ($p=0.107$). Recent ART-initiators were also more anemic (median haemoglobin 11.0 g/dl [IQR:9.0,12.0]) and had lower viral loads than the other ART categories (Figure 2). The distribution of etiologies differed substantially by ART status group ($P < 0.048$; Figure 1), in keeping with the epidemiologic evidence that the first 6 months of ART is a high risk period for stroke.[2] Within this time period, HIV-associated vasculopathy (specifically the HIV-associated vasculitis [$n=6$] and non-

atherosclerotic vasculopathy [n=3]) was the commonest diagnosis (56 %). Brain histologic material from two IRIS-like cases revealed TB Meningitis and HIV-associated vasculitis (Figure 3).

Discussion

This in-depth analysis of a large cohort of patients shows that ischemic stroke in people with HIV infection is distinct from the non-HIV population, with a younger age of onset and a different risk factor profile. Based on clinical, radiologic and autopsy analysis, we found that HIV-associated vasculopathy and opportunistic infections were the most common etiologies. Among patients with HIV-associated vasculopathy, the three subtypes (i.e. accelerated atherosclerosis, non-atherosclerosis and vasculitis) appear to have different risk factors. Importantly, most ischemic stroke patients with non-atherosclerotic vasculopathy or HIV-associated vasculitis had recently started on ART, which is suggestive of IRIS.

IRIS occurs during immune system recovery after an immune deficient state. It is associated with a rapid decline (of 2 logs or more) in HIV viral load, a low nadir and then rising CD4+ count following ART introduction, and anemia.[12] [13-15] The mechanism, although widely believed to be driven by infiltration of active T-cells, still remains unclear.[14] In our cohort, those presenting with a stroke after recently starting ART showed some risk factors for IRIS.[16] However, although HIV viral loads appeared to be largely suppressed, we did not see the higher CD4+ counts and therefore evidence of immune reconstitution typically associated with IRIS. This immune-virologic discordance was unexpected and suggests persistent immune dysregulation. Arguably, cell counts may not wholly reflect function and as evidenced by viral suppression, there may have been immune recovery even in the absence of an increased CD4+ count.[15] Such immune-virologic discordance was recently implicated in non-AIDS complications, and thus this merits further investigation.[17]

330

331 Patients diagnosed with HIV-associated vasculitis were highly immunosuppressed and thus it is
332 plausible that vessel wall inflammation was driven by an undiagnosed opportunistic infection.
333 Tuberculosis and cryptococcus, for example, are frequent triggers of central-nervous system (CNS)-
334 IRIS.[18] However, patients with confirmed opportunistic infections tended not to be on ART, and
335 had a correspondingly high HIV viral load. Furthermore, TB was only identified in 6% of ischemic
336 stroke and we did not detect cryptococcal disease. Of our 4 patients who had autopsy following
337 ischemic stroke, one patient with characteristic features of endarteritis obliterans was diagnosed
338 clinically and confirmed at autopsy with TB meningitis. Nonetheless, our results are more consistent
339 with CNS-IRIS triggered by an immune response to HIV viral antigens per se. [14] The postulated
340 mechanisms include immune response directed at residual HIV virus in the CNS, persistent release of
341 HIV-Tat protein from HIV-infected cells despite control of viral replication, and inflammatory
342 responses directed against self-antigens.[14]

343

344 Patients with atherosclerotic vasculopathy were not as immunosuppressed as patients with other
345 subtypes of HIV-associated vasculopathy. The relatively young patient ID:218, who was on ART for >
346 6 months, showed histologic evidence of extensive atherosclerosis in the absence of other vascular
347 risk factors (Supplement Figure 1). At a population level, studies in high income countries have
348 consistently shown that HIV-positive individuals have a substantially high risk of stroke, roughly
349 equivalent to that of general population cohorts 10-20 years older than themselves.[19] This is
350 despite exposure to opportunistic infections being far lower, and the additive risk of ART toxicity and
351 HIV/ART induced metabolic dysregulation (e.g. hyperlipidaemia) being accounted for.[4, 19] HIV
352 could have a causal role in this disease mechanism but this is still open to debate. However, there is
353 growing evidence that HIV-related chronic inflammation even in well-suppressed HIV infected
354 individuals is linked to sub-clinical vasculopathy.[4] Whilst HIV-associated vasculopathy appears to

be more common in Sub-Saharan Africa compared with elsewhere, the atherosclerotic and cryptogenic subtypes are likely to become the predominant subtypes as the HIV population ages and the disease stabilizes, and thus, warrants further investigation.

There were some limitations to the study, for example, we did not screen for sickle cell disease. However, although commonly associated with stroke elsewhere in sub-Saharan Africa, the prevalence of sickle cell disease in Malawi is low (<2%) and thus unlikely to have made a major contribution.[20] The absence of cerebral angiography limited our ability to refine the diagnosis of the cryptogenic group and thus further subdivide them into cardiac embolic or non-cardiac embolic causes. Indeed the latter could have represented undiagnosed HIV-associated vasculopathy. Furthermore, non-atherosclerotic vasculopathy and HIV-associated vasculitis could be manifestations of the same disease process at different stages of HIV infection, It is possible that the hospital recruitment may have been biased against milder cases in the community. It is also possible that the risk of stroke seen in those starting ART may be related to being sick and not ART itself, although difficult to tease out, our proposed mechanism of IRIS is not dissimilar to other infections, in the very immunosuppressed, such as TB and Cryptococcus CNS infection.[15, 21] Finally, CNS IRIS is associated with a poor prognosis and often fatal within days-weeks if untreated, leaving the possibility that ischemic stroke among those starting ART could have been underestimated if patients died before hospital admission.[14, 18]

HIV-associated vasculopathy and opportunistic infections are common causes of HIV-related ischemic stroke. Furthermore, subtypes of HIV-associated vasculopathy may manifest as a result of an immune reconstitution-like syndrome after starting ART. This study highlights the different phenotypes of HIV-associated vasculopathy and ties in with emerging data on neuro-inflammation

pre- and post HIV-infection. Our understanding of the underlying mechanism and the role that HIV plays is incomplete, especially on better treated cohorts with cryptogenic stroke, and possibly 'HIV-associated' atherosclerosis. This highlights the importance for future mechanistic studies to underpin the pathogenesis of these various subtypes and in time, pave the way for appropriate interventions.

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Conflict of interests

MC reports one lecture fee / meeting attendance that was sponsored by Abbvie. All other authors have no other conflict of interest.

402

403 **Author's contribution**

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405 search, analysed the data, and wrote the first draft of the paper. TS, MDC, RSH, ELC, EJ, and SL
406 revised the first draft of the paper. IT, EJ, KC, HM, SK, CS, SL, SK, MH performed the research. TS and
407 TJA supervised the conduct of the study. All authors contributed to critical revision of the
408 manuscript.

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Figure 1: Selection procedure and classification of cases

Cryptogenic* - * indicates that non-invasive or invasive angiography has not been done and therefore the subcategory of 'cryptogenic embolism' and 'other cryptogenic' cannot be determined

Figure 2: Clinical, radiologic and laboratory features among those starting ART

(A) Etiology by ART status shows HIV-associated vasculopathy to be the most common etiology among those starting ART. (B) Radiologic examples of HIV-associated vasculopathy found among those starting ART: ID 32: Diffusion weighted (left) and Fluid Attenuated Inversion Recovery (FLAIR) (middle) sequences show a left Middle cerebral artery infarct, while Doppler of the left common carotid artery (right) illustrates underlying concentric stenosis ($\geq 70\%$) extending into the bulb.; ID 278 – Middle cerebral artery infarct on Diffusion weighted (left) and T2-weighted (right) sequences; ID 46 and 85 - Diffusion weighted and FLAIR sequences show multifocal ischemic lesions in the basal ganglia and cortices. (C) Risk factors of immune reconstitution syndrome compared across the ART groups. Kruskal-Wallis non-parametric ANOVA was used to compare continuous variables across the ART treatment status groups. ART – antiretroviral treatment, CD4+ count – CD4+ T-lymphocyte count, IRIS – Immune Reconstitution Inflammatory Syndrome, CTE – Cardio-thromboembolism, O.I – Opportunistic infections, APS – Anti-phospholipid syndrome, Cryptogenic* - * indicates that non-invasive or invasive angiography has not been done and therefore the subcategory of 'cryptogenic embolism' and 'other cryptogenic' cannot be determined.

Figure 3: Radio-histologic characteristics in patients presenting with HIV-associated vasculitis versus vasculitis related to TB meningitis after starting ART

1.a-e; A 32 year old (5 months pregnant) female on ART for less than 6 months with an acute right arm monoparesis, dysphasia and headache. Her CD4+ count was 175 cells/mm³ and HIV blood and CSF viral load were undetected on admission. Mild pleocytosis (White cell count 10 cells/mm³), moderately elevated protein (1.6mg/L) and a glucose ratio of 0.48 was found on CSF examination. MRI 1.a-1.c confirmed an acute middle cerebral artery infarct. Histopathology (1.d-e) Multiple infarction of the cortical laminar type, marked periarteritis with foci of muscle necrosis, present in all sized arteries. There was lymphocytic meningitis but no granuloma or caseation or giant cells typical of TB meningitis. There was no CMV inclusion bodies and varicella zoster intrathecal IgG was negative. 2.a-e; A 34 year old female on ART for less than 6 months with an acute right arm weakness, headache, neck ache and fever. CD4+ count was 128 cells/mm³ and HIV blood and CSF viral load on admission were 1.48 and 3.22 log₁₀ copies/ml respectively. There was no CSF pleocytosis but a markedly elevated protein of 16.6mg/L and CSF glucose ratio of 0.28. MRI brain confirmed an acute infarct of the basal ganglia with mild hydrocephalus. Histopathology (2.d-e) Endarteritis obliterans of the small arteries with and a recent infarct of the basal ganglia. There was widespread meningeal inflammation with confluent and discrete tuberculoid granulomas, typical caseating necrosis and Langhans' giant cells. There were superficial Rich foci (i.e. tuberculous cerebritis adjacent to the meninges). Acid fast bacilli stain was negative but histology was characteristic of TB meningitis

Supplement Figure 1: Histologic illustration of atherosclerotic vasculopathy:

A 50 year old man on ART for greater than 6 months with an acute left hemiparesis. His CD4+ count was 192 cells/mm³ and HIV blood and CSF viral load were undetected on admission. He had no pleocytosis, a mildly elevated protein (0.9 mg/L) and a glucose ratio of 0.48 on CSF examination. A comprehensive etiological screen was unremarkable. Histopathology showed an acute infarct in the right cerebral hemisphere, and extensive atherosclerosis in all sized vessels, in both the left and right cerebral hemispheres. Sections of the right carotid artery had substantial atheroma. There was no acute thrombus. There was no HIV-associated encephalitis.

Table 1: Clinical, and radiologic characteristics of ischemic stroke in HIV positive and negative cohorts

	HIV positive (n=64)	HIV negative (n=107)	P value [‡]
Median Age (IQR)	40 (32,51)	66 (53,77)	<0.001
Male sex (%)	29 (45)	49 (46)	1.000
Family history	9 (14)	17 (17)	0.403
Hypertension (%)	27 (42)	89 (83)	<0.001
Diabetes (%)	2 (3)	14 (13)	0.032
Hypercholesterolemia (%)	4 (7)	10 (10)	0.510
Current smoker (%)	6 (9)	26 (24)	0.016
Recent infection (%)	12 (19)	8 (8)	0.082
ETOH intake (%)	13 (21)	14 (13)	0.379
Cannabis use (%)	1 (2)	3 (3)	1.000
Obesity (%)			
T1	14 (22)	17 (16)	0.496
T2	23 (37)	33 (31)	
T3	26 (41)	56 (53)	
Median ankle brachial index	1.01 (0.96,1.01)	1.01 (0.94,1.06)	0.946
Previous TIA	4 (6)	7 (7)	0.274
Previous Stroke	4 (6)	15 (14)	0.244
Radiologic Characteristics			
Acute/ subacute MRI lesions			
Cerebral cortex	39 (68)	61 (66)	0.721
Cerebellum	4 (7)	3 (3)	0.297
Brainstem	6 (11)	9 (10)	0.866
Basal ganglia	39 (68)	39 (42)	0.002
Periventricular white matter disease	24 (42)	33 (36)	0.418
Other***	1 (2)	2 (2)	0.162
More than one focal lesion (%)	12 (21)	6 (8)	0.034
Stroke characteristics			
Median NIH stroke scale	12 (8,14)	11 (7,18)	0.813
Etiology of Stroke (TOAST)			
Large artery disease	14 (21)	14 (10)	
Cardio-thromboembolism	4 (6)	13 (9)	
Small vessel disease	1 (1)	3 (2)	<0.001
Stroke of other determined [∞]	31 (46)	15 (11)	
Stroke of undetermined	18 (27)	92 (67)	
Hospital Fatality	11 (17)	10 (9)	0.152

** Cerebral cortex includes frontal, temporal, occipital and parietal lobe

***Corpus callosum, hypothalamus, pituitary, cranio-cervical junction

[‡] Categorical variables were analyzed with Fisher's two-sided exact test. Kruskal-Wallis non-parametric ANOVA was used to compare continuous variables

[∞]Stroke of *Other determined cause* in the HIV negative group includes; 7 – Probable antiphospholipid syndrome, 4- Syphilis, 2 –Varicella zoster, 1 – TB, 1- Probable vasculitis.

[†]See Table 3 for HIV-associated stroke

T=Tertile

cp/ml=copies/ml

Cryptogenic* - * indicates that non-invasive or invasive angiography has not been done and therefore the subcategory of 'cryptogenic embolism' and 'other cryptogenic' cannot be determined.

Table 2: Clinical features of the different etiologies found in HIV-related ischemic stroke†

	HIV-associated vasculopathy‡ N=23			Opportunistic Infections N=16	Anti-phospholipid syndrome N=6	Cardio-thrombo embolism N=4	Cryptogenic* stroke n=11	P value¥
	Atherosclerotic vasculopathy (n=7)	Non- atherosclerotic vasculopathy (n=7)	HIV-associated vasculitis (n=9)					
Median age (IQR)	60 (50,68)	33 (24,42)	35 (32,42)	35 (28,41)	42 (32,52)	58 (48,69)	44 (31,54)	<0.001
Male (%)	4 (57)	4 (57)	4 (44)	5 (31)	4 (67)	2 (50)	2 (50)	0.606
ART status (%)								
Untreated	4 (57)	4 (57)	2 (22)	12 (75)	4 (67)	2 (50)	7 (64)	0.048
<6 months on treatment	0	3 (43)	6 (67)	3 (19)	2 (33)	1 (25)	1 (9)	
≥6 months on treatment	3 (43)	0	1 (11)	1 (6)	0	1 (25)	3 (27)	
CD4+ T-lymphocyte count mm³	271 (192,318)	248 (218,305)	88 (15,117)	131 (61,294)	93 (63,159)	302 (240,558)	204 (51,458)	0.031
HIV blood viral load log₁₀ cp/ml	3.1 (0,4.4)	3.7 (1.5,4.3)	0 (0,2.5)	3.5 (2.4,4.6)	4.7 (2.0,5.3)	1.5 (0,4.0)	1.5 (0,4.6)	0.183
Haemoglobin (g/dl)	12.0 (9.0,15.0)	11.0 (9.0,15.0)	12.0 (10.0,14.0)	12.0 (10.0,13.0)	10.0 (9.0,12.0)	14.0 (13.0,15.0)	12.0 (9.0,13.0)	0.720
NIH stroke scale	12 (7,14)	11(8,17)	13(12,18)	13 (8,16)	10 (7,12)	9 (8,11)	11 (6,14)	0.596
Hospital fatality	2 (29)	1 (14)	2 (22)	3 (19)	1 (17)	0	2 (18)	0.955

†Continuous variable presented as median with interquartile ranges

‡ Small vessel disease (n=1), Multifactorial stroke (n=1) and Inconclusive (n=2) were not included in the analysis.

¥ Categorical variables were analyzed with Fisher's two-sided exact test. Kruskal-Wallis non-parametric ANOVA was used to compare continuous variables

ART – antiretroviral treatment

Cryptogenic* - * indicates that non-invasive or invasive angiography was not done and therefore the subcategory of 'cryptogenic embolism' and 'other cryptogenic' cannot be determined

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1 **Title:**

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3 The role of HIV-associated vasculopathy in the etiology of ischemic stroke

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5 **Running Title:**

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7 Etiology of HIV ischemic stroke

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9 [40-word summary:](#)

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11 [The etiologic spectrum of stroke is different in those with HIV infection compared to those without.](#)

12 [HIV-associated vasculopathy was the commonest mechanism found. We describe an immune](#)

13 [reconstitution syndrome-like vasculopathy among those starting ART.](#)

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Abstract

Background: HIV infection is a recognized risk factor for young stroke but the exact mechanisms are poorly understood. We studied the clinical, radiologic and histologic features of HIV-related ischemic stroke to gain insight into the disease mechanisms.

Methods: We conducted a prospective, in-depth analysis of adult ischemic stroke patients presenting to Queen Elizabeth Central Hospital, Blantyre, Malawi, in 2011.

Results: We recruited 64 HIV positive and 107 HIV negative patients. Those with HIV were significantly younger ($p < 0.001$) and less likely to have established vascular risk factors. Patients with HIV were more likely to have large artery disease (21% versus 10%; $p < 0.001$). The commonest etiology was HIV-associated vasculopathy (24 [38%]), followed by opportunistic infections (16[25%]). Sixteen out of 64 (25%) had a stroke soon after starting antiretroviral therapy (ART), suggesting an immune reconstitution-like syndrome. In this group CD4+ T-lymphocyte count was low, despite a significantly lower HIV viral load in those recently started on treatment ($p < 0.001$).

Conclusions: HIV-associated vasculopathy and opportunistic infections are common causes of HIV-related ischemic stroke. Furthermore, subtypes of HIV-associated vasculopathy may manifest as a result of an immune reconstitution-like syndrome after starting ART. A better understanding of this mechanism may point towards new treatments.

Key words: Stroke, Vasculopathy, HIV, Africa, Immune reconstitution syndrome

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Introduction

Stroke incidence in low-to-middle income countries is increasing, especially in young populations.[1]
In many of these regions, HIV is prevalent, and younger populations are more likely to have
infectious causes of stroke.[2]

We recently showed that HIV infection makes a major contribution to the overall stroke burden
(Population Attributable ~~PAF~~ Fraction 15%) in Malawi.[2] It was the second leading risk factor
overall (behind hypertension), and the most important among young stroke patients (Population
Attributable Fraction ~~PAF~~ 42%). Starting antiretroviral (ART) treatment appeared to contribute to
stroke risk in the very immunosuppressed, but the mechanism of this is unknown.[2] Previous
reports have shown that opportunistic infections, coagulopathy and cardio-thromboembolism are
important etiologies to consider.[3] In addition, HIV infection may directly lead to HIV-associated
vasculopathy via inflammatory intermediaries.[4] The term vasculopathy is defined as intimal hyperplasia
more than expected for age, and thus encompasses several pathologic phenotypes of stroke found in HIV
infection including, 1) HIV associated accelerated atherosclerosis, 2) non-atherosclerotic vasculopathy
(patients have non-vasculitic abnormalities, with intimal hyperplasia that can progress to stenosis or
aneurysmal dilatation) 3) HIV-associated vasculitis, and 4) small vessel disease.[5] Our understanding
of the pathologic mechanisms of these phenotypes is incomplete. We have previously described more
detailed clinico-pathologic classification of HIV-associated vasculopathy.[5]

Here we report the clinical, laboratory, radiologic and autopsy features of HIV ischemic stroke
patients, explore how they differ from the non-HIV ischemic stroke population, and consider the
mechanisms of stroke among those starting antiretroviral therapy (ART).

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Methods

Participants

The study was conducted at the Queen Elizabeth Central Hospital, Blantyre Malawi; a large government hospital for much of Southern Malawi. The national prevalence of HIV in adults is 10.6% but higher (20%) in Blantyre.[6] Adults (age ≥ 18 years) who presented to the hospital within seven days of symptom onset, and met the WHO case definition of stroke '[a clinical syndrome consisting of rapidly developing clinical signs of focal \(or global in case of coma\) disturbance of cerebral function lasting more than 24 hours or leading to death with no apparent cause other than a vascular origin.](#)'[7] were recruited to the study between February 2011 and April 2012 (Figure 1).

Procedure and etiological definitions

~~Baseline clinical assessment has been described elsewhere.~~[2] Clinical features, and potential vascular risk factors (i.e. age, gender, family history, ART use, hypertension, diabetes, hypercholesterolemia, acute infection, abdominal obesity, alcohol, smoking, substance use, ~~previous and previous~~ stroke/transient ischemic attack) were recorded. Stroke severity at baseline was assessed with the National Institutes of Health Stroke Scale ([NIH stroke scale](#)~~NIHSS~~), and performed within 7 days of symptom onset.[8, 9] Evidence of peripheral vascular disease was determined by measuring ankle brachial index using a handheld vascular Doppler (HI Dop; Ana Wiz Ltd UK).[10] Magnetic Resonance Imaging (MRI) of the brain was performed within 7-days of admission. The definitions for risk factors, severity of stroke and imaging protocol were previously reported.[2]

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207 Investigations included full blood count, total cholesterol, random glucose, HIV-1 serology and viral
 208 load, CD4+ T-lymphocyte count (CD4+ count) using previously described methods.[2] [HIV-1 RNA load](#)
 209 [was measured using the Hologic Aptima HIV-1 Quant Dx assay \(Hologic Inc, Manchester, UK\). This](#)
 210 [sensitive assay has a lower limit of quantitation \(LLOQ\) of 30 copies/ml and a limit of detection of 13](#)
 211 [copies/ml. Undetectable The limit of detection for HIV viral load was defined as \$\leq 1.5 \log_{10} 50\$](#)
 212 [copies/ml \(Hologic Commercial kits, Sussex, UK\).](#) Antiphospholipid syndrome diagnostics (anti-
 213 cardiolipin antibody, lupus anti-coagulant, anti- β_2 -glycoprotein 1; Cambridge Life Sciences Ltd,
 214 Cambridgeshire, UK), and specific infection screening (1. serum syphilis treponemal immunoassay +
 215 agglutination test and non-treponemal tests, and if positive cerebrospinal fluid [CSF] venereal
 216 disease research laboratory test, and 2. monoclonal intrathecal varicella zoster[VZV]-antibody
 217 determination)[11] were not done locally and thus, performed using standard protocols, at the
 218 haematology department, Royal Liverpool Hospital UK, and Public Health England respectively. All
 219 blood cultures and CSF diagnostics [i.e. microscopy, biochemistry, India ink and acid fast bacilli
 220 stains, cryptococcal antigen, standard bacterial culture, Mycobacterium tuberculosis (TB) culture]
 221 were performed locally at the Malawi-Liverpool-Wellcome Trust (MLW) laboratory. MLW laboratory
 222 participates in internationally recognised quality control programmes including [NEQAS \(UK National](#)
 223 [External Quality Assessment Service\)](#) and the South African [National Health Laboratory Service NHLS](#)
 224 scheme. Chest X-ray, electrocardiogram, carotid/vertebral duplex ultrasound and echocardiography
 225 were also performed. When possible, a brain-only autopsy was performed in deceased HIV positive
 226 patients. Brain tissue was stored in 10% formalin and processed at the University of Edinburgh, UK.
 227 The tissue sections were stained with hematoxylin and eosin and Ziehl-Neelsen stain. Additional
 228 staining included p24 antigen (for HIV) and CD8, CD68 and CD3 antibodies (for inflammation). The

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results were interpreted by a neuropathologist and general pathologist with expertise in HIV

infection. Although the pathologists were not blinded to the HIV status, a consensus had to be

reached among these senior pathologists, with the third (HIV pathologist) arbitrating when needed.

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The etiology was determined using the Trial of Org 10172 in Acute Stroke Treatment (TOAST)

classification (Figure 1). To expand the other determined and undetermined category and handle

multiple etiologies in the HIV cohort, we also used the HIV Etiology for ischemic Stroke classification

(HES), as described previously (Figure 1). The physician that determined the final diagnosis was

blinded to ART status, degree of immunosuppression and HIV viral burden.

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Ethical consideration

The study was approved by the Liverpool School of Tropical Medicine, UK and the College of

Medicine Research Ethics Committee, University of Malawi. All participants or guardians gave

written informed consent.

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Statistical analysis

Discrete variables were reported as absolute counts and percentages. Continuous variables as the

median with inter-quartile ranges. HIV viral load below the lower limit of quantitation detection limit

were coded as 530 copies/ml (the assays detection quantification limit). This was then log

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[transformed to compare the median HIV viral load across specified groups.](#) -Contingency tables comparing 1) HIV positive/negative, and 2) HIV etiology for stroke classification, were analyzed with Fisher's two-sided exact test. Kruskal-Wallis non-parametric ANOVA was used to compare continuous variables. Statistical analyses were done with STATA 11.2. and GraphPad Prism version 6, GraphPad Software Inc., California, USA. A significance level of <0.05 was used throughout.

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Results

Three hundred patients with suspected stroke were screened during the study period, of whom 171 (64 HIV positive and 107 HIV negative) had ischemic strokes and met the entry criteria (Figure 1). Table 1 describes the demographic and clinical characteristics of the HIV positive and negative individuals. The 64 HIV-infected ischemic stroke cases form the basis of this study. Of these, 26 (40%) were on ART and four had brain autopsy.

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Comparison between HIV positive and negative patients

Compared with the HIV-negative patients, the HIV positive patients were significantly younger (median 40-years versus 66-years; $p < 0.001$), and less likely to be hypertensive (42% versus 89%; $p < 0.001$; Table 1). Other vascular risk factors including diabetes, hypercholesterolemia, and being a smoker, was more common in patients who were HIV negative. Imaging analysis showed that basal ganglia ischemia occurred more often with HIV ischemic stroke (68% versus 42%; $p < 0.001$). Using the TOAST classification, the other determined and undetermined etiologic category were common in both groups. However, for the better characterised categories, large artery disease had a different distribution and occurred more frequently in the HIV positive group (21% versus 10%; Table 1).

Etiology of HIV-related ischemic stroke

HIV-associated vasculopathy (i.e. accelerated atherosclerosis, non-atherosclerotic vasculopathy, HIV-associated vasculitis and small vessel disease) was the commonest etiology (38%), followed by opportunistic infections (25%); the latter included VZV, TB, and syphilis (Table 2). Although VZV was the most frequent infection, it was often not clinically obvious with only 3/9 (30%) having had a vesicular rash in the corresponding cranial distribution within 6 months of their stroke. No patient had evidence of occult cryptococcal disease. Despite a comprehensive work-up, a specific cause was not determined in the 17% with cryptogenic stroke.

Age ($p < 0.001$), CD4+ count ($p = 0.031$), and ART status ($p = 0.048$) differed significantly for the different etiologic groups. For example, non-atherosclerotic vasculopathy, HIV-associated vasculitis,

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opportunistic infection and cryptogenic stroke had a median age ≤ 45 yrs (i.e. young stroke) whereas those with atherosclerotic vasculopathy and cardio-thromboembolism were older (Table 3). In the most immunosuppressed patients whose median CD4+ count was < 200 cell/mm³, HIV-associated vasculitis, opportunistic infections and antiphospholipid syndrome were the most frequently found etiologies. The ART status differed significantly for these patients: 67% of those with HIV-associated vasculitis had started ART in the 6 months prior to their stroke; in contrast, only 33% of those with antiphospholipid syndrome, and 19% of those with opportunistic infections had recently started ART (Table 3). Furthermore, blood HIV viral load differed across these groups, being high (median 3.5 and 4.7 copies/ml respectively) in patients with opportunistic infections and antiphospholipid syndrome, in contrast to ~~being below the lower limit of quantitation~~~~being below the limit of detection,~~ ~~undetectable viral load~~ in patients with HIV-associated vasculitis. There were no significant differences in measured CSF cell count and biochemistry across the groups.

HIV-associated vasculopathy

The HIV-associated vasculopathy subtypes included accelerated atherosclerosis (n=7), non-atherosclerosis (n=7) and HIV-associated vasculitis (n=9); the median age for these patients was 60-years, 33-years and 35-years respectively (Table 3). Because there was only one case of small vessel disease, this was not included in the detailed analysis. The various types of HIV-associated vasculopathy differed by ART status and CD4+ count (Table 3); for example, no patient with atherosclerotic vasculopathy had started ART in the last six months, compared with 43% of the non-atherosclerotic and 67% of the HIV-associated vasculitis subtypes.

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At autopsy (Supplement Figure 1), two patients showed extensive atherosclerosis in all sized vessels (the images were consistent), one was on ART and young (50-years), with no established vascular risk factors. Although the other patient was older (74-years), not on ART, with a new diagnosis of hypertension; the degree of atherosclerosis was marked. All patients with HIV-associated vasculitis had a median CD4+ count of <200cells/mm³, and HIV-1 RNA were below the lower limit of quantitation below the limit of detection undetectable or low for HIV viral load; this differed from those with non-atherosclerotic and atherosclerotic vasculopathy (Table 3).

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Initiating ART

Sixteen out of 64 (25%) patients had an ischemic stroke within 6 months of starting ART. Ten (63%) of these recent ART-initiators had a stroke within 1 month of starting ART. The median age, CD4+ count and blood HIV viral load were 37-years (IQR:31,47), 122 cells/mm³ (IQR:73,237) and 1.5 log₁₀ copies/ml (IQR:0.7,2.1) respectively. We explored established risk factors for immune reconstitution inflammatory syndrome (IRIS), such as anemia, low CD4+ count, and a drop in HIV viral load. Patients recently started on ART had the lowest median CD4+ count (122 [IQR: 73,236] compared to 159 [IQR: 65,279] in patients never started on ART, and 295 [IQR: 192,455] in patients on ART for ≥6 months (p=0.107). Recent ART-initiators were also more anemic (median haemoglobin 11.0 g/dl [IQR:9.0,12.0]) and had lower viral loads than the other ART categories (Figure 2). The distribution of etiologies differed substantially by ART status group (P <0.048; Figure 1), in keeping with the

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epidemiologic evidence that the first 6 months of ART is a high risk period for stroke.[2] Within this time period, HIV-associated vasculopathy (specifically the HIV-associated vasculitis [n=6] and non-atherosclerotic vasculopathy [n=3]) was the commonest diagnosis (56 %). Brain histologic material from two IRIS-like cases revealed TB Meningitis and HIV-associated vasculitis (Figure 3).

Discussion

This in-depth analysis of a large cohort of patients shows that ischemic stroke in people with HIV infection is distinct from the non-HIV population, with a younger age of onset and a different risk factor profile. Based on clinical, radiologic and autopsy analysis, we found that HIV-associated vasculopathy and opportunistic infections were the most common etiologies. Among patients with HIV-associated vasculopathy, the three subtypes (i.e. accelerated atherosclerosis, non-atherosclerosis and vasculitis) appear to have different risk factors. Importantly, most ischemic stroke patients with non-atherosclerotic vasculopathy or HIV-associated vasculitis had recently started on ART, which is suggestive of IRIS.

IRIS occurs during immune system recovery after an immune deficient state. It is associated with a rapid decline (of 2 logs or more) in HIV viral load, a low nadir and then rising CD4+ count following ART introduction, and anemia.[12] [13-15] The mechanism, although widely believed to be driven by infiltration of active T-cells, still remains unclear.[14] In our cohort, those presenting with a stroke after recently starting ART showed some risk factors for IRIS.[16] However, although HIV viral loads appeared to be largely suppressed, we did not see the higher CD4+ counts and therefore evidence of immune reconstitution typically associated with IRIS. This immune-virologic discordance was

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unexpected and suggests persistent immune dysregulation. Arguably, cell counts may not wholly reflect function and as evidenced by viral suppression, there may have been immune recovery even in the absence of an increased CD4+ count.[15] Such immune-virologic discordance was recently implicated in non-AIDS complications, and thus this merits further investigation.[17]

Patients diagnosed with HIV-associated vasculitis were highly immunosuppressed and thus it is plausible that vessel wall inflammation was driven by an undiagnosed opportunistic infection. Tuberculosis and cryptococcus, for example, are frequent triggers of central-nervous system (CNS)-IRIS.[18] However, patients with confirmed opportunistic infections tended not to be on ART, and had a correspondingly high HIV viral load. Furthermore, TB was only identified in 6% of ischemic stroke and we did not detect cryptococcal disease. Of our 4 patients who had autopsy following ischemic stroke, one patient with characteristic features of endarteritis obliterans was diagnosed clinically and confirmed at autopsy with TB meningitis~~TBM~~. Nonetheless, our results are more consistent with CNS-IRIS triggered by an immune response to HIV viral antigens per se. [14] The postulated mechanisms include immune response directed at residual HIV virus in the CNS, persistent release of HIV-Tat protein from HIV-infected cells despite control of viral replication, and inflammatory responses directed against self-antigens.[14]

Patients with atherosclerotic vasculopathy were not as immunosuppressed as patients with other subtypes of HIV-associated vasculopathy. The relatively young patient ID:218, who was on ART for > 6 months, showed histologic evidence of extensive atherosclerosis in the absence of other vascular

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risk factors (Supplement Figure 1). At a population level, studies in high income countries have consistently shown that HIV-positive individuals have a substantially high risk of stroke, roughly equivalent to that of general population cohorts 10-20 years older than themselves.[19] This is despite exposure to opportunistic infections being far lower, and the additive risk of ART toxicity and HIV/ART induced metabolic dysregulation (e.g. hyperlipidaemia) being accounted for.[4, 19] [HIV could have a causal role in this disease mechanism but this is still open to debate. However, there is growing evidence that HIV-related chronic inflammation even in well-suppressed HIV infected individuals is linked to sub-clinical vasculopathy. Whether HIV has a causal role is still open to debate however. There is growing evidence that the HIV-related chronic inflammation that accompanies even in well-suppressed HIV infected individuals infection, may play a critical an important role in this mechanism.](#) [4] Whilst HIV-associated vasculopathy appears to be more common in Sub-Saharan Africa compared with elsewhere, the atherosclerotic and cryptogenic subtypes are likely to become the predominant subtypes as the HIV population ages and the disease stabilizes, and thus, warrants further investigation.

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There were some limitations to the study, for example, we did not screen for sickle cell disease. However, although commonly associated with stroke elsewhere in sub-Saharan Africa, the prevalence of sickle cell disease in Malawi is low (<2%) and thus unlikely to have made a major contribution.[20] The absence of cerebral angiography limited our ability to refine the diagnosis of the cryptogenic group and thus further subdivide them into cardiac embolic or non-cardiac embolic causes. Indeed the latter could have represented undiagnosed HIV-associated vasculopathy. Furthermore, non-atherosclerotic vasculopathy and HIV-associated vasculitis could be manifestations of the same disease process at different stages of HIV infection, It is possible that the

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hospital recruitment may have been biased against milder cases in the community. It is also possible that the risk of stroke seen in those starting ART may be related to being sick and not ART itself, although difficult to tease out, our proposed mechanism of IRIS is not dissimilar to other infections, in the very immunosuppressed, such as TB and Cryptococcus CNS infection. [15, 21] Finally, CNS IRIS is associated with a poor prognosis and often fatal within days-weeks if untreated, leaving the possibility that ischemic stroke among those starting ART could have been underestimated if patients died before hospital admission. [14, 18]

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HIV-associated vasculopathy and opportunistic infections are common causes of HIV-related ischemic stroke. Furthermore, subtypes of HIV-associated vasculopathy may manifest as a result of an immune reconstitution-like syndrome after starting ART. This study highlights the different phenotypes of HIV-associated vasculopathy and ties in with emerging data on neuro-inflammation pre- and post HIV-infection. Our understanding of the underlying mechanism and the role that HIV plays is incomplete, especially on better treated cohorts with cryptogenic stroke, and possibly 'HIV-associated' atherosclerosis. This highlights the importance for future mechanistic studies to underpin the pathogenesis of these various subtypes and in time, pave the way for appropriate interventions.

A better understanding of the mechanisms of this vasculopathy may point towards new treatments and thus, help to reduce the burden of stroke in this relatively young population.

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Author's contribution

LAB, TS, TJA, RSH, ELC and MDC designed the study. LAB performed the research, did the literature search, analysed the data, and wrote the first draft of the paper. TS, MDC, RSH, ELC, EJ, and SL revised the first draft of the paper. IT, EJ, KC, HM, SK, CS, SL, SK, MH performed the research. TS and TJA supervised the conduct of the study. All authors contributed to critical revision of the manuscript.

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Figure 1: Selection procedure and classification of cases

Cryptogenic* - * indicates that non-invasive or invasive angiography has not been done and therefore the subcategory of 'cryptogenic embolism' and 'other cryptogenic' cannot be determined

Figure 2: Clinical, radiologic and laboratory features among those starting ART

(A) Etiology by ART status shows HIV-associated vasculopathy to be the most common etiology among those starting ART. (B) Radiologic examples of HIV-associated vasculopathy found among those starting ART: ID 32: Diffusion weighted (left) and Fluid Attenuated Inversion Recovery (FLAIR) (middle) sequences show a left Middle cerebral artery infarct, while Doppler of the left common carotid artery (right) illustrates underlying concentric stenosis ($\geq 70\%$) extending into the bulb; ID 278 - Middle cerebral artery infarct on Diffusion weighted (left) and T2-weighted (right) sequences; ID 46 and 85 - Diffusion weighted and FLAIR sequences show multifocal ischemic lesions in the basal ganglia and cortices. (C) Risk factors of immune reconstitution syndrome compared across the ART groups. Kruskal-Wallis non-parametric ANOVA was used to compare continuous variables across the ART treatment status groups.

ART - antiretroviral treatment, CD4+ count - CD4+ T-lymphocyte count, IRIS - Immune Reconstitution Inflammatory Syndrome, CTE - Cardio-thromboembolism, O.I - Opportunistic infections, APS - Anti-phospholipid syndrome, Cryptogenic* - * indicates that non-invasive or invasive angiography has not been done and therefore the subcategory of 'cryptogenic embolism' and 'other cryptogenic' cannot be determined.

Figure 3: Radio-histologic characteristics in patients presenting with HIV-associated vasculitis versus vasculitis related to TB meningitis after starting ART

1.a-e; A 32 year old (5 months pregnant) female on ART for less than 6 months with an acute right arm monoparesis, dysphasia and headache. Her CD4+ count was 175 cells/mm³ and HIV blood and CSF viral load were undetected on admission. Mild pleocytosis (White cell count 10 cells/mm³), moderately elevated protein (1.6mg/L) and a glucose ratio of 0.48 was found on CSF examination. MRI 1.a-1.c confirmed an acute middle cerebral artery infarct. Histopathology (1.d-e) Multiple infarction of the cortical laminar type, marked periarthritis with foci of muscle necrosis, present in all sized arteries. There was lymphocytic meningitis but no granuloma or caseation or giant cells typical of TB meningitis. There was no CMV inclusion bodies and varicella zoster intrathecal IgG was negative. 2.a-e; A 34 year old female on ART for less than 6 months with an acute right arm weakness, headache, neck ache and fever. CD4+ count was 128 cells/mm³ and HIV blood and CSF viral load on admission were 1.48 and 3.22 log₁₀ copies/ml respectively. There was no CSF pleocytosis but a markedly elevated protein of 16.6mg/L and CSF glucose ratio of 0.28. MRI brain confirmed an acute infarct of the basal ganglia with mild hydrocephalus. Histopathology (2.d-e) Endarteritis obliterans of the small arteries with a recent infarct of the basal ganglia. There was widespread meningeal inflammation with confluent and discrete tuberculoid granulomas, typical caseating necrosis and Langhans' giant cells. There were superficial Rich foci (i.e. tuberculous cerebritis adjacent to the meninges). Acid fast bacilli stain was negative but histology was characteristic of TB meningitis

Supplement Figure 1: Histologic illustration of atherosclerotic vasculopathy:

A 50 year old man on ART for greater than 6 months with an acute left hemiparesis. His CD4+ count was 192 cells/mm³ and HIV blood and CSF viral load were undetected on admission. He had no pleocytosis, a mildly elevated protein (0.9 mg/L) and a glucose ratio of 0.48 on CSF examination. A comprehensive etiological screen was unremarkable. Histopathology showed an acute infarct in the right cerebral hemisphere, and extensive atherosclerosis in all sized vessels, in both the left and right cerebral hemispheres. Sections of the right carotid artery had substantial atheroma. There was no acute thrombus. There was no HIV-associated encephalitis.

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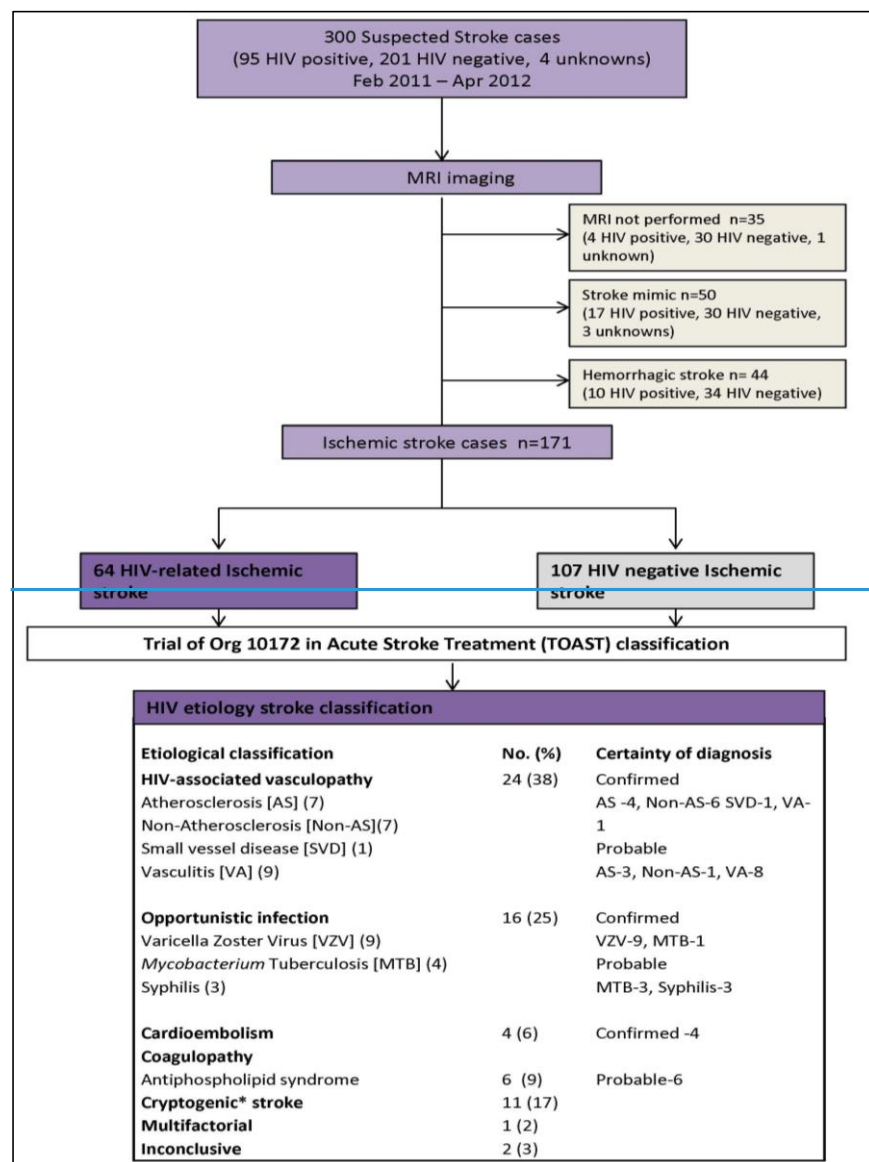


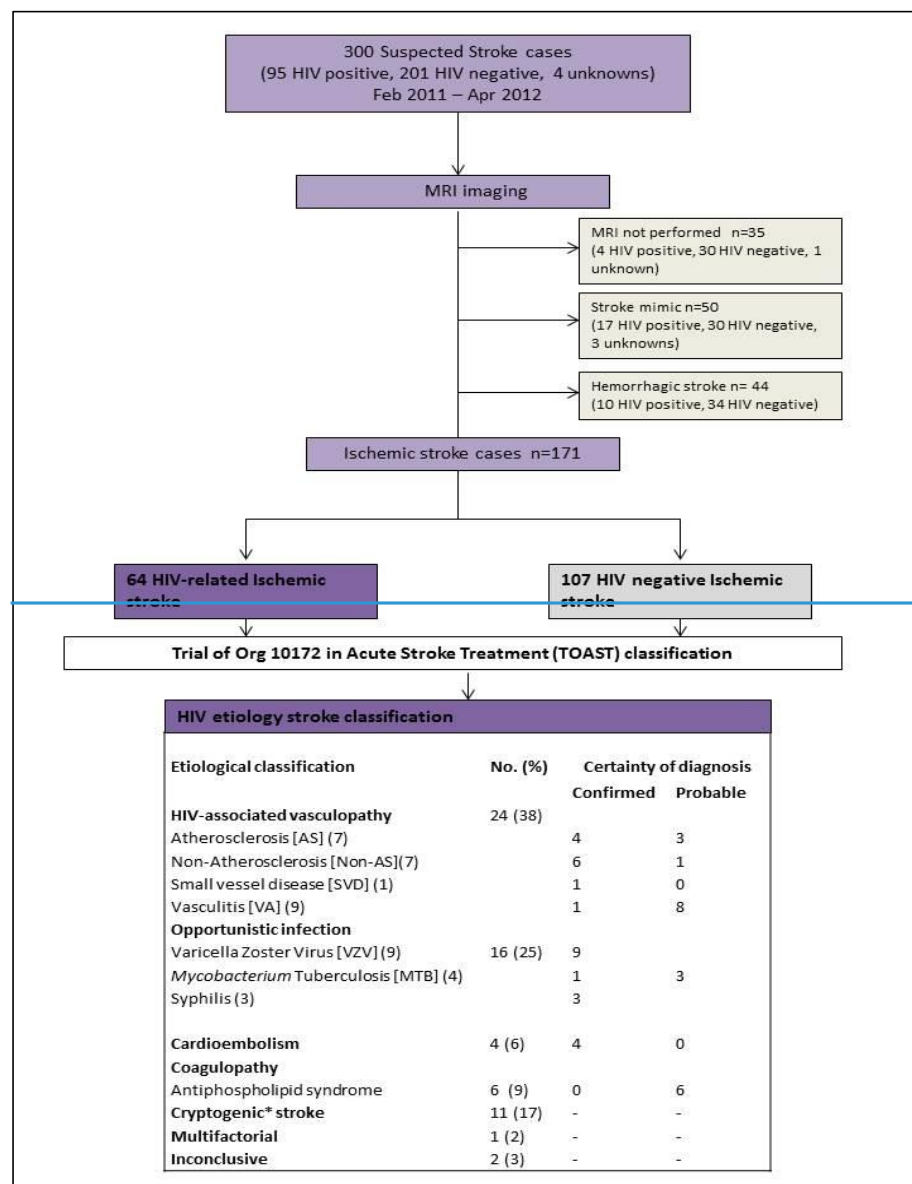
Figure 1- Selection procedure and classification of cases

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Table 1: Clinical, and radiologic characteristics of ischemic stroke in HIV positive and negative cohorts			
	HIV positive (n=64)	HIV negative (n=107)	P value [‡]
Median Age (IQR)	40 (32,51)	66 (53,77)	<0.001
Male sex (%)	29 (45)	49 (46)	1.000
Family history	9 (14)	17 (17)	0.403
Hypertension (%)	27 (42)	89 (83)	<0.001
Diabetes (%)	2 (3)	14 (13)	0.032
Hypercholesterolemia (%)	4 (7)	10 (10)	0.510
Current smoker (%)	6 (9)	26 (24)	0.016
Recent infection (%)	12 (19)	8 (8)	0.082
ETOH intake (%)	13 (21)	14 (13)	0.379
Cannabis use (%)	1 (2)	3 (3)	1.000
Obesity (%)			
T1	14 (22)	17 (16)	0.496
T2	23 (37)	33 (31)	
T3	26 (41)	56 (53)	
Median ankle brachial index	1.01 (0.96,1.01)	1.01 (0.94,1.06)	0.946
Previous TIA	4 (6)	7 (7)	0.274
Previous Stroke	4 (6)	15 (14)	0.244
Radiologic Characteristics			
Acute/ subacute MRI lesions			
Cerebral cortex	39 (68)	61 (66)	0.721
Cerebellum	4 (7)	3 (3)	0.297
Brainstem	6 (11)	9 (10)	0.866
Basal ganglia	39 (68)	39 (42)	0.002
Periventricular white matter disease	24 (42)	33 (36)	0.418
Other***	1 (2)	2 (2)	0.162
More than one focal lesion (%)	12 (21)	6 (8)	0.034
Stroke characteristics			
Median NIH stroke scale NIHSS	12 (8,14)	11 (7,18)	0.813
Etiology of Stroke (TOAST)			
Large artery disease	14 (21)	14 (10)	<0.001
Cardio-thromboembolism	4 (6)	13 (9)	
Small vessel disease	1 (1)	3 (2)	
Stroke of other determined [∞]	31 (46)	15 (11)	
Stroke of undetermined	18 (27)	92 (67)	
Hospital Fatality	11 (17)	10 (9)	0.152

** Cerebral cortex includes frontal, temporal, occipital and parietal lobe

***Corpus callosum, hypothalamus, pituitary, cranio-cervical junction

‡ Categorical variables were analyzed with Fisher's two-sided exact test. Kruskal-Wallis non-parametric ANOVA was used to compare continuous variables

[∞]Stroke of Other determined cause in the HIV negative group includes; 7 – Probable antiphospholipid syndrome, 4- Syphilis, 2 –Varicella zoster, 1 – TB, 1- Probable vasculitis.

†See Table 3 for HIV-associated stroke

T=Tertile

cp/ml=copies/ml

Cryptogenic* - * indicates that non-invasive or invasive angiography has not been done and therefore the subcategory of 'cryptogenic embolism' and 'other cryptogenic' cannot be determined.

Version 24

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Corresponding Author: Laura Benjamin, Institute of Infection and Global Health, University of Liverpool, L69 7BE

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Table 2: Clinical features of the different etiologies found in HIV-related ischemic stroke†

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	HIV-associated vasculopathy* N=23			Opportunistic Infections N=16	Anti-phospholipid syndrome N=6	Cardio-thrombo embolism N=4	Cryptogenic* stroke n=11	P value‡
	Atherosclerotic vasculopathy (n=7)	Non- atherosclerotic vasculopathy (n=7)	HIV-associated vasculitis (n=9)					
Median age (IQR)	60 (50,68)	33 (24,42)	35 (32,42)	35 (28,41)	42 (32,52)	58 (48,69)	44 (31,54)	<0.001
Male (%)	4 (57)	4 (57)	4 (44)	5 (31)	4 (67)	2 (50)	2 (50)	0.606
ART status (%)								
Untreated	4 (57)	4 (57)	2 (22)	12 (75)	4 (67)	2 (50)	7 (64)	0.048
<6 months on treatment	0	3 (43)	6 (67)	3 (19)	2 (33)	1 (25)	1 (9)	
≥6 months on treatment	3 (43)	0	1 (11)	1 (6)	0	1 (25)	3 (27)	
CD4+ T-lymphocyte count mm ³	271 (192,318)	248 (218,305)	88 (15,117)	131 (61,294)	93 (63,159)	302 (240,558)	204 (51,458)	0.031
HIV blood viral load log ₁₀ cp/ml	3.1 (0,4.4)	3.7 (1.5,4.3)	0 (0,2.5)	3.5 (2.4,4.6)	4.7 (2.0,5.3)	1.5 (0,4.0)	1.5 (0,4.6)	0.183
Haemoglobin (g/dl)	12.0 (9.0,15.0)	11.0 (9.0,15.0)	12.0 (10.0,14.0)	12.0 (10.0,13.0)	10.0 (9.0,12.0)	14.0 (13.0,15.0)	12.0 (9.0,13.0)	0.720
NIH stroke scale	12 (7,14)	11 (8,17)	13 (12,18)	13 (8,16)	10 (7,12)	9 (8,11)	11 (6,14)	0.596
Hospital fatality	2 (29)	1 (14)	2 (22)	3 (19)	1 (17)	0	2 (18)	0.955

† Continuous variable presented as median with interquartile ranges

‡ Small vessel disease (n=1), Multifactorial stroke (n=1) and Inconclusive (n=2) were not included in the analysis.

§ Categorical variables were analyzed with Fisher's two-sided exact test. Kruskal-Wallis non-parametric ANOVA was used to compare continuous variables

ART – antiretroviral treatment

Cryptogenic* - * indicates that non-invasive or invasive angiography was not done and therefore the subcategory of 'cryptogenic embolism' and 'other cryptogenic' cannot be determined.

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≥6 months on treatment	3 (43)	0	1 (11)	1 (6)	0	1 (25)	3 (27)	
CD4+ T lymphocyte count mm ³	271 (192,318)	248 (218,305)	88 (15,117)	131 (61,294)	93 (63,159)	302 (240,558)	204 (51,458)	0.031
HIV blood viral load log ₁₀ cp/ml	3.1 (0,4.4)	3.7 (1.5,4.3)	0 (0,2.5)	3.5 (2.4,4.6)	4.7 (2.0,5.3)	1.5 (0,4.0)	1.5 (0,4.6)	0.183
Haemoglobin (g/dl)	12.0 (9.0,15.0)	11.0 (9.0,15.0)	12.0 (10.0,14.0)	12.0 (10.0,13.0)	10.0 (9.0,12.0)	14.0 (13.0,15.0)	12.0 (9.0,13.0)	0.720
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†Continuous variable presented as median with interquartile ranges
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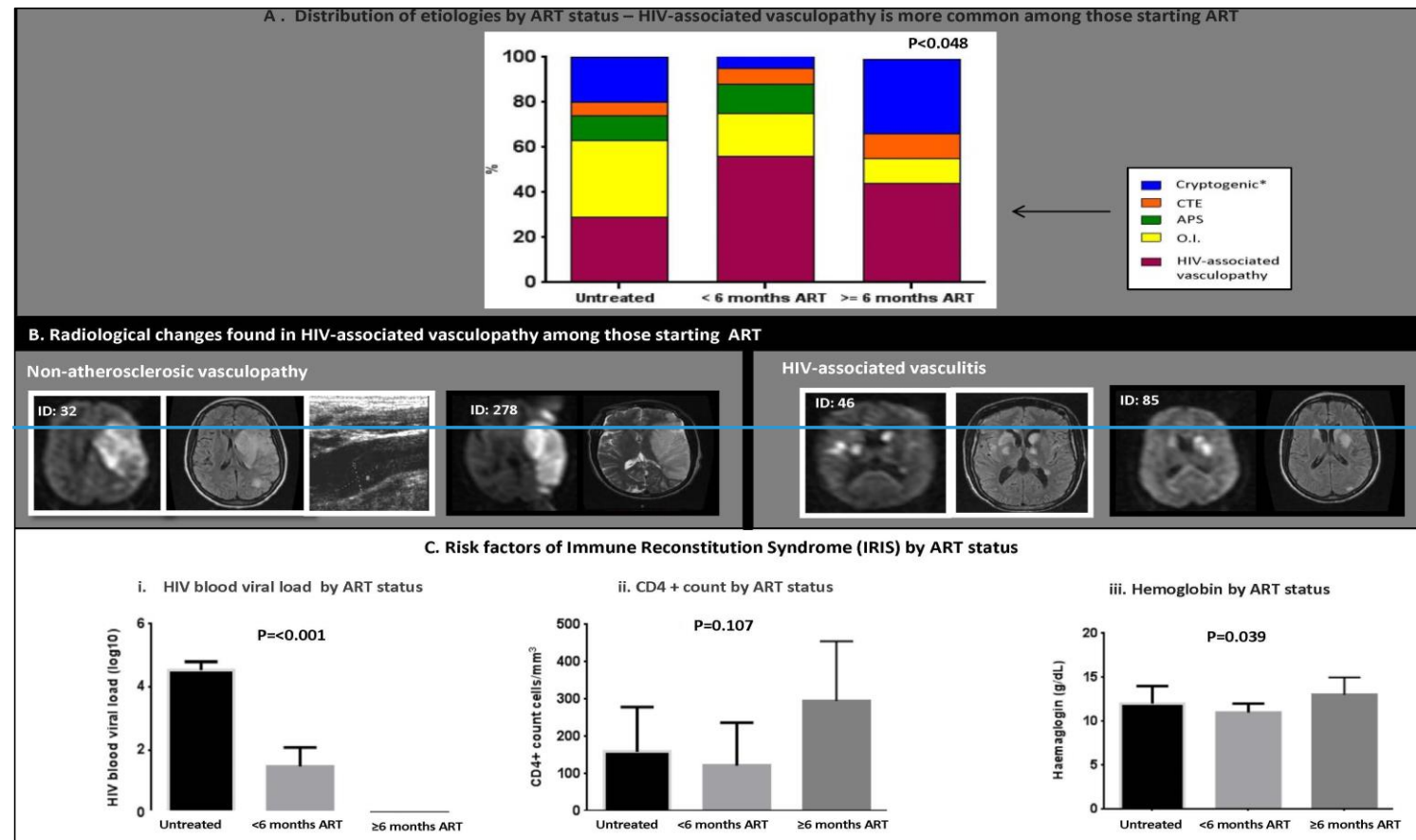
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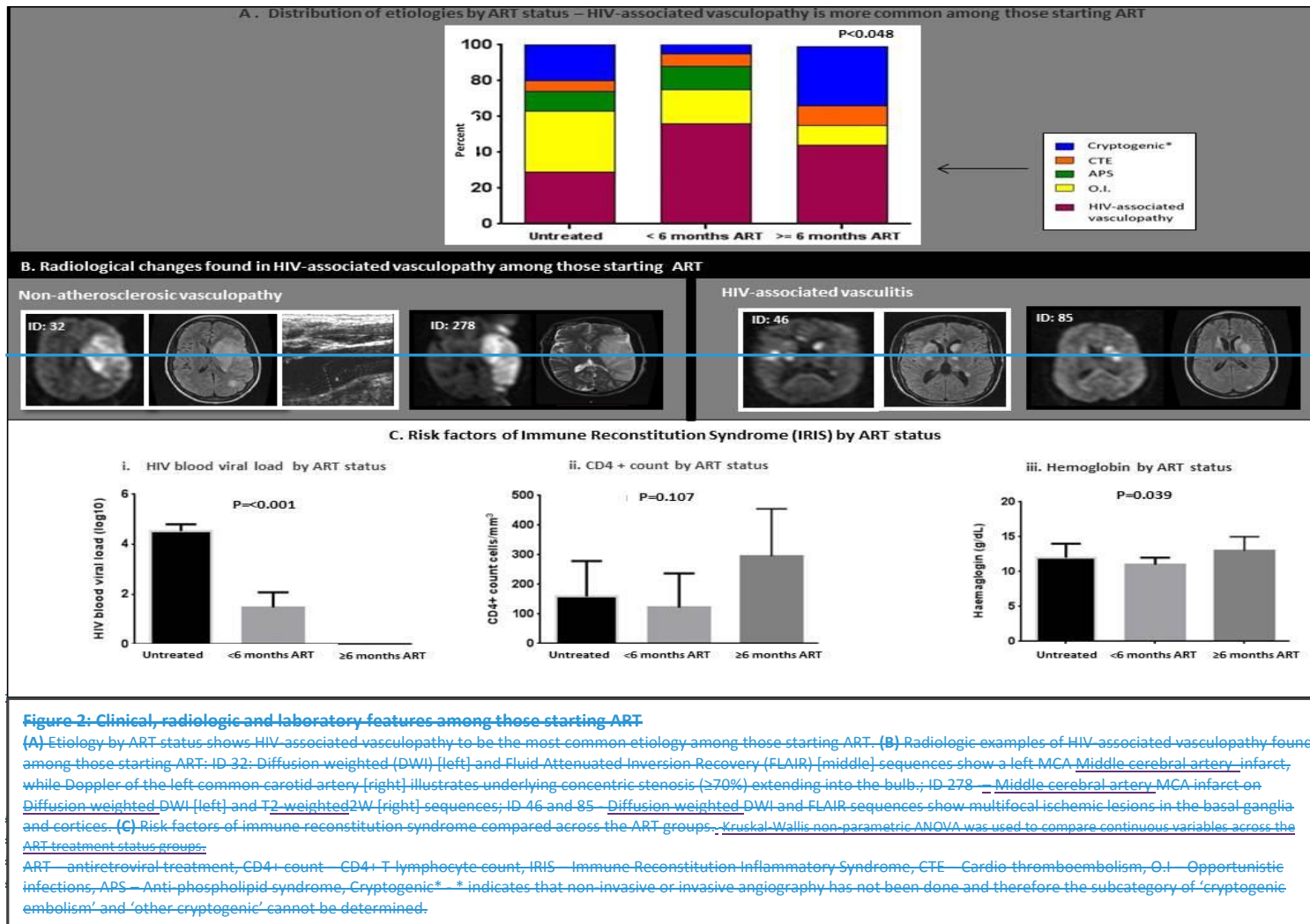
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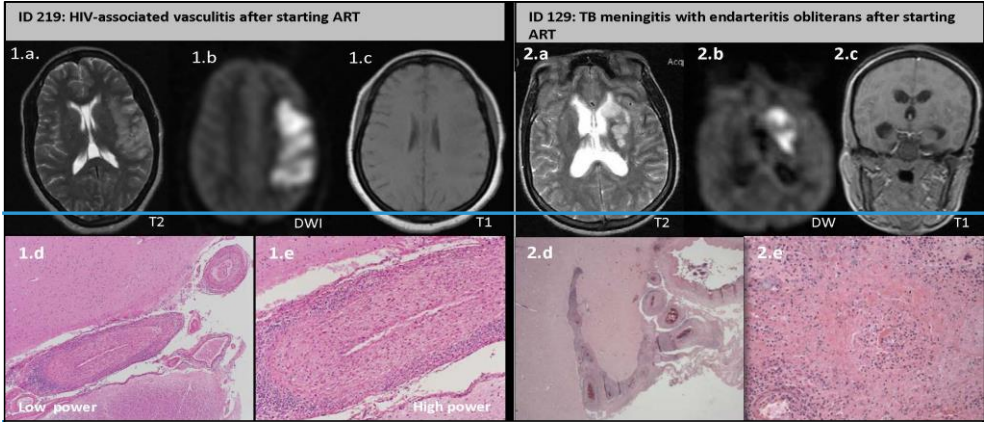
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Figure 3: Radio-histologic characteristics in patients presenting with HIV-associated vasculitis versus vasculitis related to TB meningitis after starting ART

1.a-e: A 32-year-old (5 months pregnant) female on ART for less than 6 months with an acute right arm monoparesis, dysphasia and headache. Her CD4+ count was 175 cells/mm³ and HIV blood and CSF viral load were undetected on admission. Mild pleocytosis (White cell count CC 10 cells/mm³), moderately elevated protein (1.6 mg/L) and a glucose ratio of 0.48 was found on CSF examination. MRI 1.a-1.c confirmed an acute middle cerebral artery infarct. Histopathology (1.d-e) Multiple infarction of the cortical laminar type, marked periaarteritis with foci of muscle necrosis, present in all-sized arteries. There was lymphocytic meningitis but no granuloma or caseation or giant cells typical of TB meningitis. Tuberculous meningitis (TBM). There was no CMV inclusion bodies and varicella zoster intrathecal IgG was negative. **2.a-e:** A 34-year-old female on ART for less than 6 months with an acute right arm weakness, headache, neck ache and fever. CD4+ count was 129 cells/mm³ and HIV blood and CSF viral load on admission were 1.48 and 3.22 log₁₀ copies/ml respectively. There was no CSF pleocytosis but a markedly elevated protein of 16.6 mg/L and CSF glucose ratio of 0.28. MRI brain confirmed an acute infarct of the basal ganglia with mild hydrocephalus. Histopathology (2.d-e) Endarteritis obliterans of the small arteries with and a recent infarct of the basal ganglia. There was widespread meningeal inflammation with confluent and discrete tuberculoid granulomas, typical caseating necrosis and Langhans' giant cells. There were superficial Rich foci (i.e. tuberculous cerebritis adjacent to the meninges). Acid fast bacilli stain was negative but histology was characteristic of TB meningitis **TBM**.

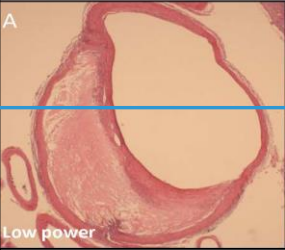
NIHSS – National Institutes of Health Stroke Scale
CXR – Chest x-ray
TBM – Mycobacterium Tuberculosis meningitis
ECG – electrocardiogram
RCC – Red cell count

APS – Antiphospholipid syndrome
TB – Mycobacterium Tuberculosis
VZV – Varicella zoster virus
WCC – White cell count
CRAG – cryptococcal antigen

no other conflict of interest.
Kingdom Medical Research Council Fellowship (T.S.)

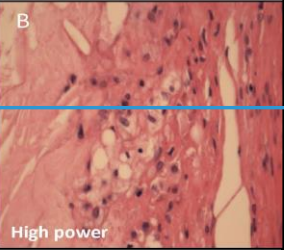
ID 218: Atherosclerotic vasculopathy

A



Low power

B



High power

Supplement Figure 1: Histologic illustration of atherosclerotic vasculopathy:

A 50 year old man on ART for greater than 6 months with an acute left hemiparesis. His CD4+ count was 192 cells/mm³ and HIV blood and CSF viral load were undetected on admission. He had no pleocytosis, a mildly elevated protein (0.9 mg/L) and a glucose ratio of 0.48 on CSF examination. A comprehensive etiological screen was unremarkable. Histopathology showed an acute infarct in the right cerebral hemisphere, and extensive atherosclerosis in all sized vessels, in both the left and right cerebral hemispheres. Sections of the right carotid artery had substantial atheroma. There was no acute thrombus. There was no HIV-associated encephalitis.

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Dear Drs Hirsch and Kovacs,

RE: JID Submission MS# 61556 - The role of HIV-associated vasculopathy in the etiology of ischemic stroke

Your revised manuscript has been improved and is nearly ready for acceptance. There is one point that needs to be addressed in a further revision.

1. In figure 1, the value for all opportunistic infections, "16 (25)" is misaligned with VZV; please correct.

Thanks for highlighting. This has now been amended.

Your revised manuscript should be submitted within two weeks of the date of this letter at our website <http://jid.edmgr.com>. In formatting your response, please provide point-by-point answers to the reviewers by copying the reviewers' comments and inserting your response underneath each comment. Please provide two copies of your revised manuscript, one "clean" and one with the changes highlighted.

Although we cannot assure you that a revised version of your manuscript will be accepted, we will process it as promptly as possible. Please remember to follow the requirements for manuscript preparation in the Instructions for Authors (at http://www.oxfordjournals.org/our_journals/jid/for_authors/)

PLEASE NOTE:

With your revision, we also need the following information. Omission of any items will delay the processing of your manuscript.

1. Each author must complete the ICMJE Uniform Disclosure Form for Potential Conflicts of Interest, available at: http://www.oxfordjournals.org/our_journals/cid/for_authors/coi_disclosure.pdf

The corresponding author is responsible for collecting the forms from each author. The completed forms must be uploaded electronically as part of the revision.

This has been done

2. Major Articles must be no longer than 3,500 words, and Brief Reports no longer than 2,000 words.

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3. Insert limits: for Major Articles, 7 in print (tables and figures, with no more than 4 panels per figure), no more than 25 MB of online-only supplementary data; for Brief Reports, 2 in print (tables and figures, with no more than 4 panels per figure), no more than 25 MB of online-only supplementary data.

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7. Source of funding and conflict of interest information, detailed for all authors, should be listed in the acknowledgment section of the manuscript.

This has been done

8. If any change is made to the author listing, such as a reordering of authors or an addition of an author, an agreement form signed by all authors must be submitted to the editorial office before the revision can be processed.

No changes have been made

9. Include on the title page of the manuscript a brief, 40-word-or-less summary of your article's main point. If accepted, this will be published under the article heading in the journal's table of contents.

This has been done

Thank you for submitting your work to The Journal of Infectious Diseases.

Sincerely,

Martin S. Hirsch, MD
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We look forward to hearing forward to hearing from you.

Yours Sincerely,

Laura Benjamin

(On behalf of all the authors)

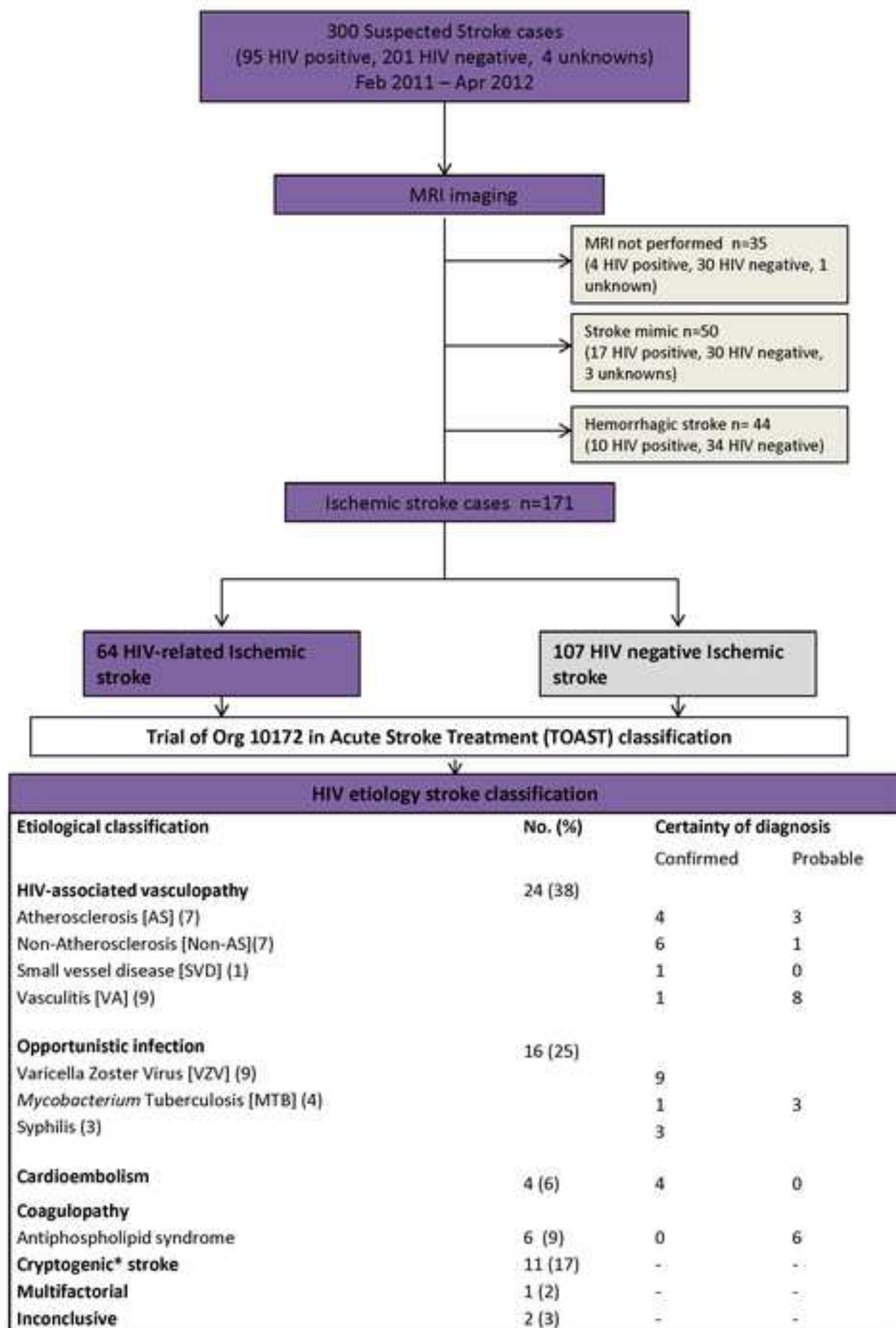


Figure 2

[Click here to download Figure 2_Imaging of less than 6 months_3.jpg](#)

